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HYDROGEN-ION STUDIES

IV. CHANGES IN REACTION ACCOMPANYING THE PRECIPITATION OF COLLOIDAL GOLD BY SPINAL FLUID (LANGE TEST) *

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The colloidal gold test with spinal fluid, commonly known as the Lange test, is an important aid in the diagnosis of cerebrospinal syphilis. The results of the test are expressed in color changes from the pink-red of the prepared colloidal gold suspension, and these in turn are plotted in graphs, or are designated by numbers (chart 1). The color of the colloidal gold suspension is dependent on the dispersion of the finely divided gold particles, and changes from the pink-red color are accompanied by an increase in the size of the aggregates.

Faraday¹ observed these color changes in his gold solutions on the addition of a salt, and correctly regarded the changes as due to an increase in the size of the gold particles. Jevons² (1868), studying the Brownian movement of particles in suspension, observed that this movement ran parallel with the stability of the suspension, and that the addition of acids, alkalis, or salts, regardless of their chemical composition caused the cessation of the movement and coagulated the suspensions. These results suggested to Jevons that the particles were electrically charged, and that the coagulating action of the electrolytes is due to the neutralization of an electrical charge carried by the particles of gold. Billitzer,³ experimenting with the electrical migration of colloidal particles of gold and other metals, found that this gradually decreases and finally changes its direction on the addition of electrolytes, demonstrating that even the sign of the charge on the colloidal particle may be changed by adding enough oppositely charged electrolyte ions.

Particles of gold in colloidal suspension carry a negative electrical charge, and ions, metallic or colloidal, precipitating them are positively charged. Burton² studied the mobility of colloidal gold particles after the addition of varying amounts of 0.001 N aluminum sulphate (Al^{+}), and the results obtained indicate clearly the presence of an iso-electric point for the gold suspensions, and that the gold particle passes through a state of maximum instability at the time its electrical charge is changing from negative to positive. After passing through the iso-electric point, an increase in the quantity of electrolyte added increases the stability of the suspension. When the smallest traces of aluminum sulphate are added to the colloidal gold, all of the aluminum ions go to decrease the charge on the gold particles, and when aluminum is added in quantities just

Received for publication March 17, 1922.

* Aided by the Winfield Peck Memorial Fund.

¹ Philosophical Mag., 1857, 4, pp. 401 and 512.

² See Burton, E. F.: The Physical Properties of Colloidal Solutions, 1916, p. 145.

³ Ann. d. Physik, 1903, 11, p. 902.

sufficient to neutralize that charge, coagulation of the particles is most rapid. If the electrolyte is added at once in excess, the particles absorb the metallic ion, and the charge is changed from a large negative to a large positive, the positive charge inducing the same stabilizing effects as the negative, and so maintaining the colloidal particle in a state of fine subdivision. Hardy⁴ suggests that the coagulation of a colloidal suspension by electrolytes takes place because the electrical charges of the particles are neutralized by the absorption of oppositely charged ions of the electrolyte solution, and at the iso-electric point where the charge becomes zero, the colloidal coagulates. Burton,² summarizing the work on the ultramicroscopic observations of electrolytic coagulation of colloids, says that electrolyte coagulation progresses by the condensation of small particles on those of larger size, and not by the coalescence of particles of equal size. The larger ultramikrons act as condensation nuclei for the smaller particles. The color changes of the gold solution follow parallel with the ultramicroscopic changes in that the size of the particles increases and their number

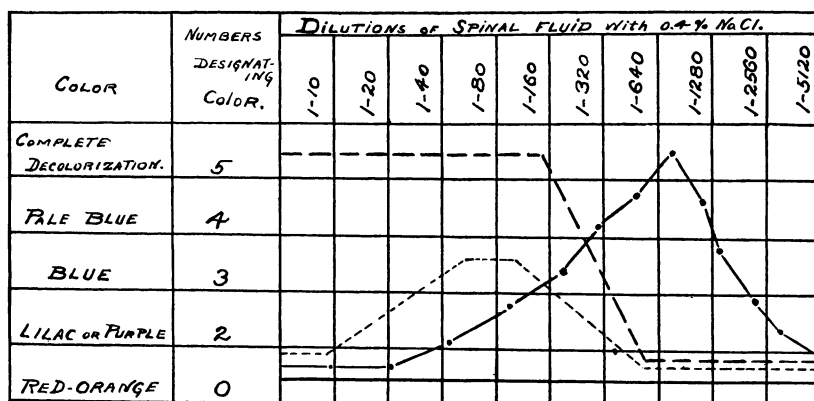


Chart 1.—Typical curves with the Lange test. The continuous line indicates a normal spinal fluid; the long dashes, a paretic spinal fluid; the short dashes, a syphilitic spinal fluid; and the dot and dash line, precipitation in higher dilutions.

decreases as more and more of the electrolyte is added. Gold solutions, as observed by Faraday,¹ Zsigmondy⁵ and others, pass through a series of color changes on the addition of coagulating agents. These are red, purple-red, red-violet, blue-violet and deep blue. The suspension becomes turbid, and finally the gold separates in the form of powder or flakes.

Certain organic colloids when added in small quantities to a metal solution prevent the coagulation of the suspended particles by electrolytes. Zsigmondy⁶ found this protection specific for each protein examined, and he expressed this relation in terms of milligrams of protein capable of protecting 5 cc of his colloidal gold solution against precipitation by 0.5 cc of a 10% sodium chlorid solution. Lange,⁷ with the possibility of clinical application in mind, after having failed with blood serum, attempted its application in a quantitative study of the

⁴ Jour. Physiol., 1905-6, 33, p. 251.

⁵ Zur Erkenntniss der Kolloide, 1905.

⁶ Ztschr. f. Anal. Chem., 1901, 40, p. 697.

⁷ Ztschr. f. Chemotherapie, O., 1912-13, 1, p. 44.

proteins of the cerebrospinal fluid. His first attempts were unsuccessful because in using distilled water to make the spinal fluid dilutions, certain proteins, particularly the globulins, were thrown out of solution. However, with a weak salt solution (0.4% sodium chlorid) the spinal fluid proteins did not precipitate, and the salt concentration was too low to agglutinate the gold particles. With this procedure, Lange obtained results which were as interesting as they were startling to him. He found that normal spinal fluid diluted with 0.4% sodium chlorid solution caused no color change with the colloidal gold, while abnormal fluids, which reasonably could be expected to protect against precipitation because of their protein content, caused partial or complete precipitation of the gold particles with resultant color changes. These, plotted in curves according to dilutions, seem to be almost specific for certain diseases, particularly syphilis. Fluids from patients with tabes or cerebrospinal syphilis reacted within a range of dilutions regularly enough to suggest the term "syphilitic curve," while fluids from certain types of meningitis reacted in such a way as to make curves characterized by the term "Verschiebung nach Oben" or reactions with the greatest precipitation in the highest dilutions. Spinal fluids from parietic patients caused complete flocculation in the first four or six dilutions, making a curve known as the "parietic curve."

Lange thought the various reactions indicated qualitative mixtures of proteins. Zaloziaki⁸ regards the changes as analogous to immunity reactions.

A previous study⁹ revealed the interesting fact that the medium in which bacteria are agglutinated by homologous immune serum becomes more alkaline, in proportion with the degree of agglutination. Bacteria suspended in dilute salt solution carry negative electrical charges, just as do the particles of colloidal gold. It seems possible, then, that the precipitation of the colloidal gold particles which occurs in the Lange test with positive syphilitic spinal fluid, may be accompanied by changes in the reaction of the medium similar to the changes occurring with the agglutination of a suspension of bacteria by homologous immune serum. The gold solution was prepared chemically¹⁰ and its reaction adjusted to approximate neutrality. The spinal fluid dilutions and mixtures with the gold solution were made in clean glass tubes as follows:

Gold Solution	5 c c	5 c c	5 c c
0.4% NaCl	0.9 c c	0.95 c c	0.975, etc.
Spinal fluid	0.1 c c	0.05 c c	0.025

These tubes stood at room temperature over night, and their reactions were determined the next morning at constant temperature (25C.) by the gaschain method. Chart 2 illustrates graphically the results

⁸ Deutsch. Ztschr. f. Nervenhe., 1913, 47, p. 783.

⁹ Jour. Infect. Dis., 1922, 30, p. 651.

¹⁰ Bull. Johns Hopkins Hosp., 1915, 26, p. 391.

of such an experiment with a spinal fluid giving a modified paretic curve. The Wassermann reaction was 100% positive with the acetone-insoluble and cholesterinized antigens. With it is plotted a normal negative control. In the table are included the results obtained with other spinal fluids.

All of these results demonstrate that the agglutination of the gold particles as manifested by color changes, turbidity or complete precipitation is accompanied by a parallel increase in the alkalinity of the medium.

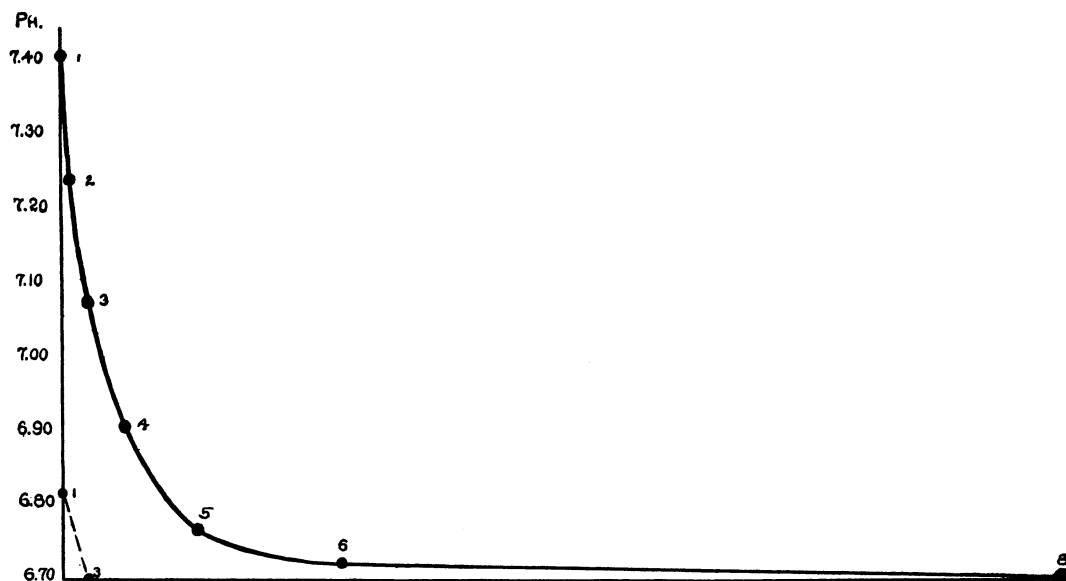


Chart 2.—Curve showing the reactions in dilutions of spinal fluid and colloidal gold giving a modified paretic curve. Complete precipitation of the colloidal gold in the first two tubes; no color change after the seventh tube.

COMMENT

The negative electrical charges of the colloidal particles of a metal such as gold are thought to arise from an incomplete chemical combination with water as follows: $\text{Au} + \text{H.OH} = (\text{Au}_{\text{..}}\text{H}^+) + \text{OH}^-$. The gold-hydrogen aggregate dissociates slightly and gives rise to a negative electrical charge on the metal. In the presence of a small amount of sodium chloride, it is possible that the dissociated Na-ions carry the positive charges in equilibrium with the negative electrical charges of the colloidal gold. The addition of syphilitic spinal fluid precipitates the

gold particles like aluminum sulphate. The reacting ion (Al) carries a positive electrical charge, and it is reasonable to conclude that the ions in the spinal fluid reacting with the gold also carry a positive electrical charge. Linder and Picton¹¹ classified colloids into anionic and cationic according to the migration of the particles to the positive or negative electrode. They observed also that under certain conditions a colloid bearing a charge of one sign is precipitated by the addition of a colloid of an opposite sign, and that both colloids are

TABLE 1
RESULTS OF THE PRECIPITATION OF A SPINAL FLUID MANIFESTING A STABILIZING EFFECT

	Precipitation in Higher Dilutions		Paretic Curve		Syphilitic Curve		Normal	
	Color Change	P _H	Color Change	P _H	Color Change	P _H	Color Change	P _H
1	Pink, some precipitate	7.61	Complete precipitate	7.49	Rose	6.92	0	6.82
2	Pink, some precipitate	7.38	Complete precipitate	7.46	Rose	6.84	0	6.82
3	Complete precipitate	7.12	Complete precipitate	7.22	Purple	6.83	0	6.71
4	Blue	6.96	Complete precipitate	7.18	Blue	6.82	0	6.70
5	Lilac	6.93	Complete precipitate	6.95	Purple	6.80	0	
6	Purple	6.87	Blue	6.74	Red blue	6.75	0	
7	0		Purple		0	6.70	0	
8	0	6.84	0		0	6.70	0	
9	0		0		0	6.70	0	
10	0	6.70	0	6.70	0	6.70	0	6.70

carried down in the precipitate. Biltz¹² suggests that the following rules govern the precipitating and precipitated colloids, mixed together quickly and treated uniformly.

If to a given colloid one of opposite sign is added in small proportion there is no precipitating action. As the quantity of the second increases, the coagulative action follows parallel until a proportion is reached which causes immediate coagulation. As this amount is still further increased, coagulation ceases, that is, there is an optimum precipitation for certain proportions, and if these favorable proportions are exceeded on either side, no precipitation occurs.

It seems, then, that the precipitation of the colloidal gold particles is dependent on the presence of positively charged ions in the spinal fluid, probably protein substances. The neutralization of the negative charges of the ionized gold-sodium aggregate by the positive charges of the substances contained in the spinal fluid liberates the base sodium, which in water dissociates to satisfy its own constant. The

¹¹ Jour. Chem. Soc., 1892, 61, p. 148; 1895, 67, p. 63; 1897, 71, p. 568; 1905, 87, p. 1906.

¹² Berichte d. Deutsch. chem. Gesellschaft., 1904, 37, p. 1095.

protein substance dissociated in water may be represented by the formula $\frac{(\text{protein}) \times (\text{OH})}{(\text{protein OH})} = K$. The dissociation constant of the sodium hydroxide is probably so much greater than that of the protein substance that when it (K_{Na}) is satisfied, the medium contains a much greater number of dissociated hydroxyl ions, and consequently is more alkaline in reaction. The amount of base liberated depends on the degree of gold precipitation.

In accordance with the observations that an excess of an oppositely charged colloid stabilizes the gold solution are those results with spinal fluids which form curves with precipitation in the higher dilutions. In the table are given the results of a spinal fluid manifesting this stabilizing effect in the first two dilutions. The alkalinity is greater than that of any others in similar dilutions. This curve in the Lange test results when a large negative charge on the gold particles is suddenly replaced by a large positive, the solution being stabilized in the lower dilutions, but precipitated in the higher dilutions.

The color in the first two dilutions of the syphilitic curve is said to be unchanged. Careful comparison, however, demonstrates that it is rose-red and that the medium often is slightly turbid. The reaction curve of fluids in such a series of tubes conforms in outline if not in length with the paretic and other curves.

CONCLUSIONS

The agglutination of colloidal gold particles by spinal fluid (Lange test) is accompanied by an increase in the alkalinity of the medium in which this reaction occurs.

This change in reaction is similar to that observed on the agglutination of bacteria by homologous immune serum.